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Cellular and molecular pathways involved in the bacterial endophyte-mediated biocontrol of *Sclerotinia sclerotiorum* in *Brassica juncea*

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Background:

Stem rot, caused by *Sclerotinia sclerotiorum* has become a serious threat to *Brassica juncea* which mandates the use of synthetic fungicides for its control as there are no genetic sources that pose complete resistance to the pathogen. Despite their effectiveness in controlling the disease, extensive use of fungicides is not a sustainable alternative due to their high economic and environmental costs. Therefore, efforts are needed to develop efficient and eco-friendly biocontrol for plant diseases. In this regard, endophytes (bacteria, fungi, or actinomycetes), which are non-pathogenic living organisms living in symbiosis with the robust plant, have been used as biopesticides on Brassica stem, significantly reducing the disease incidence of *S. sclerotiorum*, however, the mechanisms involved in the disease suppression are unclear.

Objective:

We have attempted to assess the role of bacterial endophytes in triggering defence signalling molecules in the management of stem rot in *B. juncea*. Mechanisms and pathways involved in the bacterial endophyte-mediated biocontrol of *S. sclerotiorum* were examined at the genetic level to gain a better understanding.

Methods:

A plate inhibition experiment was used to initially screen 55 endophytic bacterial isolates for their antagonistic activity against *S. sclerotiorum*. The identities of the selected strains exhibiting antagonistic action were confirmed by 16s rRNA sequencing. The inoculum of the identified bacterial strains was prepared in nutrient broth. The effectiveness of the endophytic strains alone and in consortia to increase defence signalling metabolites (enzymatic and non-enzymatic), plant growth and yield was investigated in susceptible Brassica variety through seed treatment and foliar inoculation methods in the field trials conducted at two different locations. Foliar spray with bacterial endophyte inoculum was done 48 h prior to inoculation with *S. sclerotiorum* at the 50% flowering stage. Disease index was recorded after three weeks of inoculation. The expression profiling of genes associated with oxidative stress responses in *B. juncea*, has also been carried out using qRT-PCR.

Results:

Among all endophytic bacterial isolates tested, *Pseudomonas aeruginosa* and *Serratia liquefaciens* were able to inhibit the growth of *S. sclerotiorum*. The administration of cell suspensions of bacterial isolates resulted in a considerable reduction in lesion. The isolate *P. aeruginosa* alone and in consortium performed better under the combined application method (seed treatment and foliar inoculation) to enhance the enzymatic (peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, glucanase, and chitinase) and non-enzymatic (phenols and lignin) metabolites associated with oxidative stress. Likewise, the bacterial consortium also enhanced the plant growth and yield attributes of *B. juncea*. Outcomes of RNA seq-transcriptome analysis conducted to characterize the effect of bacterial endophytes on the relative expression levels of defensive genes in the endophyte-pathogen system will be discussed.

Conclusions:

Our research's findings have helped to identify bacterial endophytic strains, understand their modes of action, for combating *S. sclerotiorum* in *B. juncea*. The cellular defence pathways identified in the study may be useful in developing Brassica varieties resistant to *S. sclerotiorum*. The identified endophytes can be used to synthesize biofungicides, which can be developed into commercial pesticides for integrated disease management, either on their own or in conjunction with chemical pesticides.